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CD4 T cells play a central role in viral immunity. They provide help for B cells and CD8 T cells and can act as effectors themselves. Despite their importance, relatively little is known about the magnitude and duration of virus-specific CD4 T-cell responses. In particular, it is not known whether both CD4 Th1 memory and CD4 Th2 memory can be induced by viral infections. To address these issues, we quantitated virus-specific CD4 Th1 (interleukin 2 [IL-2] and gamma-interferon) and Th2 (IL-4) responses in mice acutely infected with lymphocytic choriomeningitis virus (LCMV). Using two sensitive assays (enzyme-linked immunospot assay and intracellular stain) to measure cytokine production at the single-cell level, we found that both CD4 Th1 and Th2 responses were induced during primary LCMV infection. At the peak (day 8) of the response, the frequency of LCMV-specific CD4 Th1 cells was 1/35 to 1/160 CD4 T cells, and the frequency of Th2 cells was 1/400. After viral clearance, the numbers of virus-specific CD4 T cells dropped to 1/260 to 1/3,700 and then were maintained at this level indefinitely. Upon rechallenge with LCMV, both CD4 Th1 and Th2 memory cells made an anamnestic response in vivo. These results show that unlike some microbial infections in which only Th1 or Th2 responses are seen, an acute viral infection can induce a mixed CD4 T-cell response with long-term memory.

CD4 T cells play an important role in viral immunity. In viral infections such as vesicular stomatitis virus, influenza A virus, and Sendai virus, CD4 T cells help B cells secrete neutralizing antibody which facilitates viral clearance. Some antiviral CD8 T-cell responses are critically dependent upon CD4 T-cell help. These include responses against adenovirus, chronic lymphocytic choriomeningitis virus (LCMV) (24), herpes simplex virus (17), and gammaherpesvirus-68 or MHV-68 (10) infections. Even in acute LCMV infection, CD4-knockout mice (CD4-/-) mice produce two- to threefold-fewer cytotoxic T lymphocytes (CTLs) compared to CD4+/- mice during the primary response and show a gradual decline in number of antiviral memory CTLs over time (36). The importance of CD4 T cells is highlighted by the finding that mice deficient in CD4 T cells is associated with a decrease in T-helper-cell number associated with a decrease in T-helper-cell responses (CD4-depleted or CD4-/- mice) are unable to generate large numbers of CTLs and cannot control strains of LCMV which replicate quickly (24). In humans, a decrease in T-helper-cell number associated with human immunodeficiency virus infection is associated with a loss of virus-specific CD8 CTLs and an increase in viral titer and susceptibility to other infectious agents. With the exception of influenza virus and Sendai virus infections of mice (14, 32), relatively little is known about the primary burst size of virus-specific CD4 T cells following systemic viral infection and the size of the CD4 memory pool.

Furthermore, little is known about the types of CD4 T cells which develop after viral infection. After antigen stimulation, T-helper development proceeds along two paths: one leads to formation of T-helper type 1 (Th1) cells, and the other leads to Th2 cells (reviewed in references 1 and 26). Th1 cells and Th2 cells can be distinguished by differences in their cytokine profiles. Th1 cells secrete IL-2, tumor necrosis factor alpha, tumor necrosis factor beta (lymphotoxin-alpha), and gamma-interferon (IFN-gamma) and assist in activating CD8 T cells and macrophages and promote immunoglobulin G (IgG) antibody class switching to the IgG2a isotype, whereas Th2 cells make IL-4, IL-5, and IL-10 and facilitate B-cell activation and the development of IgG1 antibody. Each T-helper subset governs the other, because cytokines produced by one subset negatively regulate the production of cytokines by the other. Leishmania major infection in mice provides an example of the biological implications of these opposing T-helper cells: mice that are prone to making high levels of Th1 cytokines in response to this intracellular parasite resolve the infection, whereas mice that tend to make less IFN-gamma are susceptible (15). As another example, the autoimmune disease experimental allergic encephalomyelitis (EAE) in mice is induced by autoreactive Th1 cells (20). Induction of Th2 cells and cytokines ameliorates the disease (11, 18). While still controversial, it has been reported that human immunodeficiency virus-infected individuals switch from a Th1 to a Th2 phenotype as they progress in disease (12), and it is not known why this change occurs. It is also unknown how many Th1 and Th2 cells are generated, what mechanisms govern development of one type over the other, and the biological implications of this in acute viral infection.

In this report, we document the activation and expansion of virus-specific CD4 T cells and the longevity of CD4 T-cell memory in mice infected with LCMV. We report that by 1 week postinfection, there was expansion of virus-specific IFN-gamma-secreting CD4 T cells resulting in a frequency of 1/35 CD4 T cells, which then declined during the following 2 to 3 weeks to 1/260 CD4 T cells. This number was stably maintained for at least 250 days postinfection, and immune mice were able to...
mount accelerated secondary CD4 responses following rechallenge. Most LCMV-specific T cells were of the Th1 type, but large numbers of Th2 CD4 T cells were also generated and maintained. Given the central role of CD4 T cells in providing help for generating and maintaining memory CTL responses and B-cell responses, our data indicate that vaccine strategies which target CD4 helper cells may prove useful for increasing the numbers of these cells for protective amnestic responses against viral infections.

MATERIALS AND METHODS

Mice. C57BL/6 (H-2b) mice were purchased from Jackson Laboratory, Bar Harbor, Maine. The C57BL/6 carrier mice used in these experiments were generated and bred at Emory University as previously described (4). These mice are congenitally infected and express viral protein in the context of major histocompatibility class I (MHC I) and MHC II molecules.

Virus. The Armstrong CA 1371 strain of LCMV was used in these studies for infection of mice (5). Infectious virus in serum and liver was quantitated by plaque assay on Vero cells (5).

Flow cytometry. Spleen cells were stained with antibodies which recognize CD8 (clone 53-6.7), CD4 (clone RM4-5), and CD44 (clone IM7) with a concentration of 1.0 µg/ml/106 cells. These antibodies were purchased from Pharmingen (La Jolla, Calif.), Annexin V-fluorescein isothiocyanate (FITC), used to measure apoptotic cells, was purchased from Pharmingen, and the manufacturer’s recommended protocol was followed.

CD4 T-cell enrichment. CD4 T-cell enrichment by negative selection was done with CD4 enrichment columns. Mouse CD4 subset column kits were purchased from R & D Systems (Minneapolis, Minn.), and the manufacturer’s suggested protocol was used. CD4 T cells were >90% pure by this protocol, and the number of CD6 T cells was <0.5%, as indicated by flow cytometry. As an additional check on the levels of CD4 T-cell contamination, NP396-404, an MHC I-restricted peptide, was added to CD4 cell-enriched cultures in some experiments, and the number of virus-specific CD8 T cells was quantitated by enzyme-linked immunospot assay (ELISPOT). By this test, there was little to no detectable CD8 T-cell contamination above background levels in the CD4 T-cell-enriched cultures. The number of CD4 T cells recovered after column enrichment was 25 to 50% of the initial number loaded onto the column. Analysis of CD44 expression before and after column enrichment indicated that there was a relative loss of subset CD44hi (activated) cells during the enrichment process (data not shown).

Quantitation of virus-specific IFN-γ-secreting CD8 and CD4 T cells. Virus-specific CD8 and CD4 T-cell responses were measured by IFN-γ ELISPOT assay (13, 30) by using whole spleen cells or CD4 purified preparations from mice immunized with LCMV. The capture antibody for this assay, rat anti-mouse IFN-γ (clone XMG1.2; Pharmingen), was used at 2 µg/ml per 106 cells or 2 µg/ml per 106 cells on a cellulose-bottom plates (Millipore, France). After dilutions of effector cells were added to the plate, feeder cells (1,200-rad-irradiated uninfected mouse spleen cells) were added at 5 × 106 cells per well to maintain cell-cell contact. Effector cells were incubated for 26 to 37°C without (medium alone) or with stimulation. For stimulation, either carrier mouse spleen cells or purified LCMV peptides which bind to MHC class I and can stimulate CD8 T-cell responses specifically (25, 35) or LCMV peptides which bind to MHC class II (NP396-398 and GP61-80 of Armstrong) and stimulate only CD4 T cells were added to CD4 cell-enriched cultures in some experiments, and the number of virus-specific CD8 T cells was quantitated by enzyme-linked immunospot assay (ELISPOT) for 18 to 20 h. These antibodies were purchased from Pharimingen, and the manufacturer’s recommended protocol was followed.

RESULTS

Activation and expansion of CD4 T cells. To investigate primary CD4 T-cell responses during viral infection, mice were infected with 2 × 106 PFU of the Armstrong strain of LCMV, and T-cell responses were analyzed by flow cytometry. Consistent with previous reports (2, 6, 21, 37), large numbers of CTLs were generated, and infection was controlled by day 8 postinfection, as indicated by plaque assay of serum and liver (data not shown). Figure 1 shows that there was a shift in the number of CD4 T cells which expressed the activation marker CD44 following infection. In naive mice, most cells were CD44lo and CD44hi CD4 T cells increased from 4 × 106 per spleen at day 0 to 8 × 106 per spleen at day 8 and 13 × 106 by day 15 (Fig. 2A). This ratio changed to 1.9 by day 15 as the immune response to LCMV was subsiding and approached homeostasis by day 30 at ~0.9. Activation was also associated with an increase in cell number. The number of activated CD44hi CD4 T cells increased from 4 × 106 per spleen at day 0 to 8 × 106 per spleen at day 8 and 13 × 106 by day 15 (Fig. 2A). In contrast, the number of CD44lo CD4 T cells changed little after infection. There was a slight decrease in number at day 8, but cell numbers returned to ~7 × 106 by day 30. Figure 2B shows that CD8 T cells had a more pronounced increase in cell number in the same mice. The number of CD44hi CD8 T cells increased from 3 × 106 per naive spleen to 45 × 106 per spleen at day 8 postinfection, fivefold more than the number of activated CD4 T cells. To investigate whether the increase in cell number was due to cell division, mice were fed BrdU in their drinking water during the first week of infection, and the number of CD4 T cells which incorporated BrdU was measured by flow cytometry. Figure 3 shows that in mice responding to infection, 84% of CD44hi CD4 T cells divided during the period from day 0 to day 8, whereas only 43% of CD44hi cells from naive mice divided. CD44lo cells did not proliferate in response to infection, and the number of these cells which were BrdU positive (7%) was comparable to that found in naive mice (6%). These data indicate that there was virus-induced cell division among the CD44hi cells, and the increase in the number of activated CD4 T cells in the spleen represents expansion of T cells rather than recruitment to this site.
Development of long-term CD4 Th1 memory. To quantitate the number of virus-specific CD4 Th1 cells following infection, splenic CD4 T cells were purified by column enrichment and analyzed with IFN-γ and IL-2 ELISPOT assays following virus restimulation. The frequency of IFN-γ-secreting LCMV-specific CD4 T cells increased to 1/162 CD4 T cells by day 8 (Fig. 4A). This frequency corresponded to $1.1 \times 10^5$ virus-specific CD4 cells per spleen.

Following the peak of the CD4 response, there was a period in which 87 to 95% of the virus-specific CD4 T cells died. Annexin V staining of CD4 T cells during this period indicated that 30% to 40% of CD4 T cells were apoptotic, and 10 to 15% of CD4 T cells from naive mice were apoptotic in the same assay (data not shown). By 1 to 2 months postinfection, the frequency of IFN-γ-secreting memory CD4 T cells ranged from 1/1,100 to 1/3,246 CD4 T cells, corresponding to $4 \times 10^3$ to $3 \times 10^4$ per spleen (Fig. 4A). At 6 months postinfection, elevated numbers of IFN-γ-secreting memory Th1 CD4 cells ($5 \times 10^3$ per spleen) could be found.

The Th1 response was also quantitated at the epitope level by IFN-γ ELISPOT. Figure 5A shows that at the peak of the response (day 8), $5 \times 10^5$ CD4 T cells per spleen (1/38 CD4) recognized LCMV GP61-80 and $1.3 \times 10^5$ CD4 T cells per spleen (1/139) recognized LCMV NP309-328. In immune mice (day 150), fewer cells could be found which recognized these epitopes; however, there remained $3.1 \times 10^4$ CD4 T cells per spleen (1/356) which recognized GP61-80 and $9.0 \times 10^3$ CD4 T cells per spleen (1/1,126) which recognized NP309-328, which shows that long-term Th1 memory exists for both epitopes. Interestingly, three- to fourfold more cells recognized GP61-80 than NP309-328 during the peak of the response and during the memory phase. This suggests that during the death phase, there was no selective loss of one population of cells, because there was an ~15-fold drop in the number of CD4 T cells specific for both epitopes.

Long-term CD4 T-cell memory was also characterized by intracellular staining for IFN-γ following restimulation with NP309-328 and GP61-80. Figure 6 depicts CD4 T cells surface stained for CD44 and stained for intracellular IFN-γ. Less than 0.04% (1/2,500) of naive CD4 T cells (day 0) produced IFN-γ following stimulation (~4,400/spleen), but by day 8, 2.8% of CD4 T cells (1/36) made IFN-γ upon restimulation, for a total of $7.8 \times 10^5$ virus-specific cells per spleen. Since all of these cells were in the CD44hi subset, this corresponded to a frequency of 1/18 activated CD4 T cells. There was a decrease in the frequency of CD4 T cells which made IFN-γ at day 15 to 0.7% (1/143), and there was a drop in number to $5.9 \times 10^4$ virus-specific cells per spleen. Afterwards, CD4 memory was stable, because the percentage of virus-specific CD4 T cells changed very little from day 60 (0.3%) to day 300 (0.4%). The frequency of
memory cells per activated CD4 cell was 1/47 at day 300, which corresponded to \(4.1 \times 10^4\) per spleen. The numbers of IL-2-secreting Th1 cells followed a similar pattern, as did the IFN-\(\gamma\)-secreting cells (Fig. 4B). There were \(1.2 \times 10^4\) LCMV-specific IL-2-secreting CD4 T cells per spleen at the peak of the expansion phase at day 8. By days 15 to 30, there was a drop in number, but large numbers of LCMV-specific memory cells \(\left(4 \times 10^3\right)\) per spleen) remained during the memory phase 6 months after infection.

**Development of long-term CD4 Th2 memory.** To quantitate the number of virus-specific Th2 cells after acute infection, splenic CD4 T cells were purified and analyzed by IL-4 ELISPOT. Similar to the Th1 response, the Th2 response peaked at day 8. The frequency of LCMV-specific CD4 T cells at this time was 1/407 CD4 T cells, which corresponded to \(5 \times 10^4\) virus-specific Th2 cells per spleen (Fig. 4C).

Following the peak, there was a period (days 8 to 30) in which the number of IL-4-secreting cells decreased. After this time, substantial numbers of memory IL-4-secreting cells \(\left(2 \times 10^3\right)\) to \(6 \times 10^3\) per spleen) were stably maintained for at least 6 months postinfection (Fig. 4C).

Figure 5B shows the number of IL-4-secreting cells specific to GP61-80 and NP309-328. At day 8, \(4 \times 10^4\) to \(7 \times 10^4\) CD4 T cells per spleen \(\left(1/200\right)\) recognized LCMV GP61-80, and \(1 \times 10^4\) to \(2 \times 10^4\) CD4 T cells per spleen \(\left(1/600\right)\) recognized LCMV NP309-328. At day 150, there remained \(1 \times 10^3\) to \(3 \times 10^3\) CD4 T cells per spleen \(\left(1/400\right)\) which recognized GP61-80 and \(5 \times 10^3\) to \(7 \times 10^3\) CD4 T cells per spleen \(\left(1/1,620\right)\) which recognized NP309-328. Similar to what was found for the number of IFN-\(\gamma\)-secreting cells (Fig. 5A), there was long-term Th2 memory for both epitopes, with more of the response directed at GP61-80 than at NP309-328.

ELISA analysis of purified CD4 T cells demonstrated that IL-10-producing cells were also generated following infection. In naive mice, \(<15\ pg/ml\) was found in virus-stimulated supernatants, whereas at day 8, \(105\ pg/ml\) was made. Furthermore, spleen cells taken from mice immunized 150 days earlier produced high levels of IL-10 \(\left(180\ pg/ml\right)\) upon virus stimulation, demonstrating that long-term CD4 Th2 memory was generated after LCMV infection.

**Anamnestic responses of CD4 memory T cells upon rechallenge.** To further demonstrate the existence of large numbers

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**Figure 3.** In vivo proliferation of CD4 T cells after viral infection. BrdU incorporation by dividing T cells was measured in naive mice and mice responding to LCMV infection during the primary phase (days 0 to 8). Activated and resting CD4 T cells (top) were analyzed for BrdU incorporation (below). As indicated, 84% of activated CD4 T cells incorporated BrdU in infected mice, whereas only 43% of activated CD4 T cells were BrdU positive in naive mice. This demonstrates that CD4 T cells which became CD44hi divided after infection. In contrast, resting (CD44lo) CD4 T cells showed no change in BrdU incorporation in response to infection. The ratio of activated to unactivated CD4 T cells was lower in mice fed BrdU than in infected control mice. There is a slight loss in the number of activated cells, most likely due to low-level toxicity of this compound in cells which have incorporated it into their DNA.
of memory T-helper cells, immune mice and naive mice were challenged with LCMV strain Armstrong, and at day 3, T-cell responses were analyzed by fluorescence-activated cell sorter and ELISPOT. There were more activation and expansion of CD4 and CD8 T cells in rechallenged immune mice than in immune controls, and naive mice showed no activation at this early time point (data not shown). Figure 7 shows that while naive mice did not mount a specific response by day 3 and immune mice had elevated numbers of virus-specific CD4 T cells, rechallenged immune mice showed a 4.5-fold increase in IFN-γ-secreting CD4 T cells and an increase in IL-2-secreting memory CD4 T cells (Fig. 7A and B). The frequency of IL-4-secreting cells also increased after rechallenge (Fig. 7C). This shows that the Th1 and Th2 cells which were present in immune mice could expand in number quickly in response to reinfection.

FIG. 4. Generation and maintenance of memory CD4 T cells. Mice were sacrificed at various times postinfection with LCMV, and CD4 T cells were column purified and analyzed by cytokine ELISPOT as indicated. As can be seen, there are three clear phases of the CD4 T-cell response: activation (days 0 to 8), where virus-specific T cells expand to 4 x 10^5 to 6 x 10^5 per spleen; death (days 8 to 30), where 90% of the T cells die; and memory (days >30), where elevated numbers of virus-specific CD4 T cells remain. These features can be seen for IFN-γ-secreting CD4 T cells (A), IL-2-secreting cells (B), and IL-4-secreting cells (C). The numbers shown are frequencies of cytokine-secreting CD4 T cells per total CD4 T cells. Note that there was no decay in the number of memory CD4 T cells over time. The error bars represent standard deviations, and the limit of detection is indicated by the dashed line.

FIG. 5. Epitope-specific analysis of CD4 T-cell responses. The number of CD4 T cells responding to LCMV GP61-80 (●) or NP309-328 (○) was quantitated by IFN-γ ELISPOT at days 8 and 150 after infection (A). There was an increase in the number of cells responding to both epitopes at day 8. The frequency of GP61-80-specific CD4 T cells was 1/38, and the frequency of NP309-328-specific CD4 T cells was 1/139. Immune mice retained elevated numbers of virus-specific CD4 cells of both specificities, with 1/336 specific to GP61-80 and 1/1,126 specific for NP309-328. The number of epitope-specific CD4 T cells was also quantitated by IL-4 ELISPOT (B). At day 8, the frequency of GP61-80-specific cells was 1/200 and the frequency of NP309-328-specific cells was 1/600. At day 150, the frequencies were 1/400 for GP61-80 and 1/1,620 for NP309-328, indicating that IL-4-secreting memory cells specific to both epitopes were maintained.

DISCUSSION

This report shows that the CD4 T-cell response, like the CD8 T-cell response, has three phases following viral infection: the activation and expansion phase occurs during the first week of infection, a death phase follows the second week of infection, and a memory phase commences after 1 month and lasts for at least 300 days postinfection.

The increase in number of virus-specific CD4 T cells seen during the first week after infection was most likely due to expansion of clones of cells rather than recruitment of cells to the spleen from other sites. All of the virus-specific CD4 T cells that could make IFN-γ were CD4+ (Fig. 6), and only CD4+ cells divided during this period (Fig. 3). At day 8 after infection, high frequencies of Th1 cells were formed, as indicated by three independent assays (IL-2 ELISPOT, IFN-γ ELISPOT, and intracellular IFN-γ staining). IFN-γ ELISPOT (Fig. 5) and intracellular IFN-γ staining (Fig. 6) both indicated that the frequency of LCMV NP309-328- or GP61-80-specific cells was 1/35 to 1/40 CD4 T cells or ~1/20 activated CD4 T cells. Epitope analysis indicated that 5 x 10^5 cells per spleen were specific for GP61-80 and 1 x 10^5 were specific for NP309-328 at day 8 (Fig. 5). The total number of virus-specific IFN-γ-secreting CD4 T cells could be higher than that reported here, since only two I-A^k-restricted epitopes were used. The specificity of the CD4 response may include other epitopes which have not yet been identified.

There was a slight discrepancy in the IFN-γ ELISPOT estimate of virus-specific CD4 T cells as assessed by peptide stimulation of unenriched cells (1/30) versus virus stimulation of CD4-enriched cultures (1/162). This may represent differences in the level of antigen presentation during the in vitro culture period. Addition of peptide to the culture may have saturated the number of MHC II molecules presenting that particular peptide so that T cells were more efficiently stimulated. An alternative explanation is that some activated CD4 T cells were
lost during the column enrichment, because the percentage of CD44hi cells decreased after enrichment compared with the percentage of CD4 T cells that were CD44hi before enrichment (data not shown). Given that most of the peptide-specific cells are in this CD44hi population, the use of column-purified CD4 T cells may give an underestimate of the actual frequency.

The death phase occurred during weeks 2 to 4 postinfection. Annexin V staining indicated that there were threefold more apoptotic CD4 T cells during this period than in naive mice. The number of virus-specific IFN-γ- and IL-4-secreting CD4 cells dropped 87 to 95% during this period, mirroring what happens to the CD8 T-cell response (3, 6, 21, 25). Epitope analysis indicated that there was a similar drop in the number of NP309-328-specific CD4 T cells as there was for GP61-80-specific CD4 T cells, and both groups dropped ~15-fold between days 8 and 150. Three- to fourfold more cells were specific for GP61-80 than for NP309-328 at day 8, and this ratio remained the same in immune mice.

The CD4 T-cell memory established after 1 month was stable for 6 to 10 months postinfection. This was seen by using single-cell cytokine ELISPOT assays, ELISA analysis, and intracellular IFN-γ staining, followed by flow cytometry quantitation. All of the virus-specific CD4 T cells that persisted in immune mice were CD44hi (Fig. 6) and CD69lo (data not shown), indicating that they were memory cells and not recently activated effector cells (CD69hi). Mice were also able to mount rapid secondary CD4 T-cell responses upon reinfection with LCMV. The longevity of CD4 T-cell memory was comparable to that of CD8 T cells, but was ~10-fold lower in magnitude. The number of memory CD4 T cells may have been established by the size of the expansion phase, and since there was less expansion of CD4 cells than CD8 cells, the size of the memory pool was set lower. Studies in our laboratory (25) and in others (9) have shown that most (50 to 70%) of the activated CD8 T cells expanding after infection are specific for LCMV. The data shown in this report indicate that the expa-
sion of LCMV-specific CD8 T cells is 35-fold greater than that of LCMV-specific CD4 T cells. As can be seen in Fig. 2B, even if all of the activated CD4 T cells expanding after infection were specific for LCMV, there would still be an approximately fivefold smaller burst size for the T-helper compartment.

Th2 responses showed a pattern of expansion, death, and memory that was similar to that of the Th1 response. After infection, there was an increase in IL-4-secreting cells, which reached a peak at day 8 with $4.5 \times 10^4$ IL-4-secreting CD4 T cells per spleen (Fig. 4C). ELISA analysis indicated that IL-10-producing CD4 T cells were also generated. There was a drop in the number of IL-4-secreting cells between days 8 and 10, which was followed by a period of memory in which elevated numbers of virus-specific cells were maintained. However, there were fewer IL-4-secreting CD4 cells than IFN-γ-secreting CD4 cells at all times. As can be seen in Fig. 4, the frequency of Th1 cells was at all times ≥2.0-fold higher than that of Th2 cells. That most of the T-helper response was composed of Th1 cells is not surprising given the antibody isotype distribution. Seventy percent of the T-helper-dependent antiviral IgG antibody made is of the IgG2a isotype (37). Th2 responses lead to IgG1 isotype switching, and following LCMV infection, a smaller component (10%) of the IgG response is of this class.

Many microbial infections lead to either Th1 or Th2 CD4 T-cell responses. *Listeria monocytogenes*, *L. major*, and *Toxoplasma gondii* infections tend to induce a Th1 response; helminth infections tend to elicit Th2 responses (28). In contrast, a mixture of both Th1 and Th2 cells developed following acute LCMV infection, and both Th1 and Th2 memory existed long after infection (Fig. 4 and 7). A mixed response in cytokine production following LCMV infection has also been reported by others (29). IL-12 and IFN-γ have been shown to be important molecules for initiating and propagating Th1 development. Since LCMV is macrophage tropic, the initial antiviral Th1 response may be driven by activated macrophages, which produce IL-12 (7, 34), and NK cells, which produce IFN-γ. However, this Th1 response does not preclude Th2 development, because large numbers of IL-4-secreting cells could also be found. Also, some CD4 Th0 cells which produce both IFN-γ and IL-4 during the early stage of infection could exist. There are several potential models of how Th2 responses could develop in acutely infected mice. The Th2 response might be driven by low levels of IL-4 that were produced by antigen-specific T cells after their initial activation. According to one model, if levels of IL-4 reach a threshold, Th2 differentiation is initiated, resulting in increased IL-4 production that leads to additional Th2-cell formation (1). It is possible that after LCMV infection, this threshold was reached and resulted in a pronounced Th2 response in addition to the Th1 response.

In another model of T-helper-cell development, antigen load and level of costimulation influence the Th1 or Th2 differentiation. Th0 cells which are exposed to high antigen levels and receive high costimulation develop into Th2 cells, whereas those exposed to lower antigen levels (or low-dose infections) and with lower levels of costimulation develop into Th1 cells (8, 16, 23, 31). Since acute viral infection is a dynamic process with high antigen loads at days 2 to 4 and then lower levels of antigen afterwards (21), conditions favoring the development of each T-helper subset may vary with time after infection. T-helper-cell differentiation might also be influenced by the type of costimulation. It has been reported that B7.1 and B7.2 differentially drive Th1 or Th2 development in an EAE model of T-helper-cell development (19) and in a NOD model of autoimmunity (22). A similar mechanism might occur during LCMV infection as levels of B7.1 and B7.2 increase in the spleen (unpublished observation). Blocking studies with anti-B7.1 or anti-B7.2 antibody treatment in mice infected with LCMV should reveal whether this mechanism is important for antiviral T-helper-cell development.

Memory CD4 cells contribute to viral clearance by facilitating neutralizing antibody generation and by helping CD8 CTLs proliferate. They may also play a direct role by secreting IFN-γ to inhibit viral replication and by activating macrophages so that they are refractory to viral infection and replication. This is one of the first studies quantitating the initial burst size of the CD4 T-cell response which demonstrates long-term Th1 and Th2 memory in an acute viral infection. Future investigations will address the activation requirements and rules that govern the maintenance of memory CD4 T cells. This information may lead to improved vaccination strategies for preventing viral infections.

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